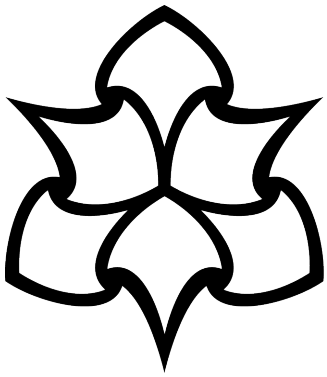

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Review

Surface modifications for antimicrobial effects in the healthcare setting: a critical overview

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SUMMARY

The spread of infections in healthcare environments is a persistent and growing problem in most countries, aggravated by the development of microbial resistance to antibiotics and disinfectants. In addition to indwelling medical devices (e.g. implants, catheters), such infections may also result from adhesion of microbes either to external solid–water interfaces such as shower caps, taps, drains, etc., or to external solid–gas interfaces such as door handles, clothes, curtains, computer keyboards, etc. The latter are the main focus of the present work, where an overview of antimicrobial coatings for such applications is presented. This review addresses well-established and novel methodologies, including chemical and physical functional modification of surfaces to reduce microbial contamination, as well as the potential risks associated with the implementation of such anti-contamination measures. Different chemistry-based approaches are discussed, for instance anti-adhesive surfaces (e.g. superhydrophobic, zwitterions), contact-killing surfaces (e.g. polymer brushes, phages), and biocide-releasing surfaces (e.g. triggered release, quorum sensing-based systems). The review also assesses the impact of topographical modifications at distinct dimensions (micrometre and nanometre orders of magnitude) and the importance of applying safe-by-design criteria (e.g. toxicity, contribution for unwanted acquisition of antimicrobial resistance, long-term stability) when developing and implementing antimicrobial surfaces.

Introduction

Infections and infectious diseases are a continuous threat to human health. According to the European Centre for Disease Prevention and Control, more than four million people are

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estimated to acquire a healthcare-associated infection (HCAI) every year in Europe (1.7 million in the USA) [1]. The number of deaths occurring as a direct consequence of these infections is estimated to be at least 37,000, and these infections are thought to contribute indirectly to an additional 110,000 deaths each year (in the USA, a total of 99,000 deaths is estimated).

The present review does not deal with indwelling medical devices. It is mostly focused on solid–air interfaces in healthcare units, such as tables, door-handles, computer keyboards, textiles, although solid–liquid surfaces are also of great concern in hospitals such as taps, showers and drains, where biofilms appear frequently. New methods, in addition or as an alternative to appropriate use of disinfectants and antibiotics, are required to reduce microbial activity, associated infections and to reverse the increase in antimicrobial resistance. A potential and promising weapon against bacterial growth and possibly the development of multidrug-resistant bacteria has been found in antimicrobial (nano)coatings (AMC) [2,3].

A state-of-the-art innovation to combat pathogenic bacteria is the creation of self-disinfecting surfaces through the application of coatings with antibiofouling and/or bactericidal properties. Bactericidal coatings are interesting in healthcare because of the capability of these coatings to kill pathogens upon contact. Many different chemical strategies and technologies for antibacterial coatings are described in the literature. For instance, antibacterial coatings may contain active eluting agents (e.g. ions or nanoparticles of silver, copper, zinc, or antibiotics, chloride, iodine), immobilized molecules that become active upon contact (e.g. quaternary ammonium polymers or peptides), or light-activated molecules (e.g. TiO_2 or photosensitizers) [4–6].

In addition to chemical modifications, the topography of a surface can by itself significantly affect its hygienic status, either in a beneficial manner (reducing microbial retention) or otherwise (increasing retention) [7]. As such, modifications of surfaces to enhance antimicrobial properties should always take into account the effect of surface wear on subsequent fouling and cleanability. Therefore, efforts should be undertaken to characterize typical wear, assess interactions with the most likely micro-organisms in that environment, and define the most appropriate and least damaging cleaning and sanitizer regimes. The best way to achieve such outcomes is to ensure that multidisciplinary expertise is integrated into developmental processes, and that testing methods are appropriately robust [8].

A relevant aspect of the surfaces is that they should be safe-by-design (SbD). In a broad sense, SbD is the elimination of the potential health and safety risks associated with a product or process by taking into account those potential risks during the early design phase. SbD is a generic concept with an ultimate goal of obtaining a product that complies with all regulations by designing out the health and safety risks that can be more difficult or sometimes impossible to deal with in the long-term after the market introduction. The SbD approach is not new in industry and has been used for many years under different names by the construction industry, aircraft industry, railway industry, etc. [9]. This opinion paper starts by assessing the different types of strategy to chemically modify the surfaces and by identifying potentially new strategies, followed by discussions on the impact of topography and the importance of the SbD approach.

Methods

Through its Cooperation in Science and Technology programme (COST), the European Commission has recently funded a four-year initiative to establish a network of stakeholders involved in development, regulation, and use of novel antimicrobial coatings for prevention of HCAI [10]. The network AMiCI (AntiMicrobial Coating Innovations) currently comprises participants of more than 60 universities, research institutes and companies across 30 European countries (www.amici-consortium.eu) and, to date, represents the most comprehensive grouping to target the use of these emergent technologies in healthcare settings. Within AMiCI, one of the working groups is collecting information on commercially available antimicrobial coatings with actual or potential application in healthcare, and the development of new coatings that are SbD. This review article is the result of extensive discussion within the working group and the AMiCI consortium as a whole, following the 'world café approach' [11].

For the identification of relevant publications, a literature search was performed in SciFinder and PubMed of studies published between 2000 and 2017. The following search terms were used, coupled with the keywords 'surface' OR 'coating': 'antimicrobial', 'antibacterial', 'functionalized', 'safe-by-design', 'topography AND (microorganism OR bacteria)', 'contact active', 'anti-adhesive', and 'biocide release'. Due to the high number of studies identified, each of the publications was then assessed for its suitability and relevance of the findings to the topic of the present review. A few publications that were not identified in the previous search, but that were known to the authors as cornerstone studies in the field of surface modifications in healthcare settings, were also included.

Chemical modifications to achieve functional antimicrobial coatings

Strategies to achieve antimicrobial coatings can be classified according to their functional principle as: (i) anti-adhesive, (ii) contact active, and (iii) biocide release (Figure 1). Whereas the first two principles may be considered as SbD, biocide release incorporates the release of a toxic substance and can therefore be considered as toxic by design. Sometimes two functional principles are combined to achieve synergistic effects, e.g. by embedding biocidal substances into anti-adhesive surfaces. Today, the majority of chemical modifications includes hydrogels or poly(ethylene glycol) (PEG) to repel approaching microbes, metals (in particular, silver and copper), antimicrobial peptides (AMPs), quaternary ammonium compounds (QACs), and nanoparticles [13–18]. Beyond those established approaches, state-of-the-art or potentially new strategies towards antimicrobial coatings were identified at the AMiCI meetings and were sorted and classified according to their functional principle. For many of the latest antimicrobial strategies, the mechanism of antimicrobial activity is still under investigation and there is not enough information available on whether antimicrobial activity happens directly at the surface or whether small amounts of active compounds are released into the test media where they will exert their antimicrobial activity, or whether both mechanisms are acting in parallel.

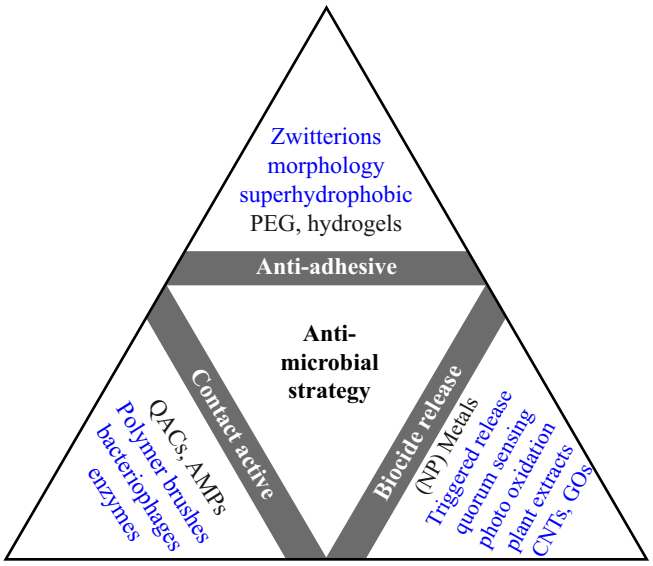

Figure 1. Established (black) and potentially upcoming strategies (blue) for antimicrobial coatings classified by their functional principle. The functional principle is also a matter of implementation, e.g. QACs are active both chemically bound to a surface and in solution. Results from the AMiCI meeting. Carbon nanotubes (CNTs), graphene(oxide)s (GOs), poly(ethylene glycol) (PEG) quaternary ammonium compounds (QACs), antimicrobial proteins peptides (AMPs), nanoparticle (NP).

Anti-adhesive surfaces

Anti-adhesive surfaces can reduce the adhesion force between bacteria and a solid surface to enable the easy removal of bacteria before a biofilm layer is formed on the surface [19]. Such surfaces may suppress HCAI by blocking transmission paths involving surfaces, but they will not reduce the number of germs on the contacting media by killing them. Attachment of bacteria or cells starts with an initial adsorption of proteins on to the material surface [20]. Strategies to prevent protein attachment include superhydrophobic surfaces, often augmented by a hierarchical nanostructure as well as zwitterionic polymers [19,21].

Superhydrophobic surfaces are characterized by a water contact angle $>150^{\circ}$ and they are inspired by the lotus leaf in nature [22]. It was further revealed that the lotus leaf has a hierarchical micro/nanostructure [23]. Reducing bacterial adhesion via superhydrophobicity is a relatively new topic and has yet to be studied thoroughly and systematically [19]. Analysis of superhydrophobic siloxane and fluorosiloxane surfaces showed also minimal protein adsorption, both before and after protein adsorption trials [24].

Nanostructures are important, since effective air entrapment in the three-dimensional nanomorphology (nanopillars) renders them superhydrophobic and slippery. On inherently nanostructured hydrophilic aluminium, adhesion forces of bacteria were reduced by a factor of 4 down to 2–4 nN compared to the electropolished flat surface, resulting in an 88% reduction of colony-forming units (cfu) for *Staphylococcus aureus*. This effect was even more pronounced after applying a hydrophobic Teflon coating, yielding a 99.9% reduction under flow conditions [25].

Nanostructured surfaces were also prepared using electrospun polystyrene nanofibres. When oxygen plasma-treated, a superhydrophilic surface was generated, which exhibited limited *Escherichia coli* attachment due to negative zeta potential of −40 mV. After fluorination, a superhydrophobic surface was obtained, which exhibited self-cleaning ability against bacteria, where the initially adhered bacteria were effectively removed with subsequent washing [26]. Anti-adhesion and killing was achieved by combining an upper superhydrophobic surface layer (silane coated poly(acrylic acid)) with limited bacterial adhesion and self-cleaning properties with a hydrophilic bottom layer (poly(ethyleneimine)–Ag^{+} complex) which could deliver bactericidal silver ions [27].

An interesting anti-adhesive and killing approach is found in nature. The nanopatterned cicada wing surface uses an adsorption and stretching mechanism with eventual rupture. As the bacterial cells adsorb on to the nanopillared structures present on the wing surfaces, the bacterial cell membrane stretches in the regions suspended between the pillars. If the degree of stretching is sufficient, cell rupture will occur [28].

Zwitterionic polymer brushes may also delay or even prevent microbial attachment to a surface, since the hydration layer surrounding the ionic surface prevents non-specific protein adsorption [21]. Using barnacle cement, a biological adhesive from barnacles, and 'click' chemistry, poly(2-(methacryloyloxy)ethyl trimethylammonium chloride) polymer brushes were successfully attached to stainless steel and antimicrobial properties were demonstrated [21]. Zwitterionic polymer brushes cannot inactivate bacterial cells. Therefore, synergistic anti-adhesion and bacterial inactivation was achieved by grafting zwitterionic poly(sulfobetaine methacrylate) brushes with embedded biocidal silver nanoparticles [29]. The importance of anti-adhesive properties for biofilm formation was also demonstrated by measuring the adhesive forces on brush-coated silicone rubber and uncoated silicon rubber. On the brush-coated rubber, adhesion was so weak that the bacteria were no longer able to sense the surface and therefore remained in their planktonic state, susceptible to antibiotics rather than forming a protected biofilm [30].

Contact-active surfaces

Contact-active surfaces exhibit antimicrobial activity without releasing biocidal substances. Several mechanisms are believed to take place in contact-active surfaces [31]. These are: (i) a so-called spacer effect, where the biocidal group is attached to the surface through a polymer chain, allowing the biocide to reach the cytoplasmic membrane of the bacteria and to perforate them; (ii) alternatively, positively charged QACs, e.g. 3-aminopropyl trimethoxysilane grafted to cellulose nanofibres, can detach phospholipids from the cell membrane and thereby kill the bacteria [32–34]. This approach is also referred to as biomimetic with respect to the activity of chitosan – a polysaccharide derived from exoskeleton of crustaceans or cell walls of fungi. Hydrophobic parts of a surface can act similarly to QACs by deforming the membrane through adhesion [35].

Polymer brushes have been widely used in preparing contact-active antimicrobial surfaces without biocidal release. The rationale behind polymer brushes is the observation that antimicrobial molecules lose much of their activity, once attached to a surface. When providing an anchor for the active

molecule through a flexible covalently bound polymeric chain, the active molecule should still be able to reach the site of action at or within the bacterium, e.g. by penetrating its cell wall, but leaching is still suppressed. Important parameters for polymer brush anchors are chain length and chain density. Polymer brushes have been shown to be effective for anchoring QACs or AMPs [36–40]. Using surface-initiated atom transfer radical polymerization, QACs with charge densities of $>1.5–10^{15}$ accessible quaternary amine units/cm^2 were anchored through poly-2-(dimethylamino)ethyl methacrylate chains. Interestingly, these surfaces were bioactive even though the polymer chains were too short to penetrate the cells with envelope thicknesses of 46 nm for Gram-negative *E. coli* and 45.55 nm for Gram-positive *Bacillus subtilis* [36]. This demonstrates that surface charge density can be more important than chain length. On the other hand, it was clearly shown that *N*-alkyl-pyridinium exhibited high antimicrobial activity when anchored through a 750 or 25 kDa polyethyleneimine (PEI) but showed no activity when using the 2 kDa analogue [37]. Therefore, only long-chained, moderately hydrophobic immobilized polycations exhibit microbicidal activity. Interestingly, polycationic polymer brushes are not subject to existing mechanisms of resistance such as multidrug-resistance pumps or multidrug tolerance protein-expressing cells, presumably since there are no analogue structures in nature [37].

Polymer brushes have also successfully been used for anchoring AMPs. AMPs are a logical alternative to conventional antibiotics due to their broad-spectrum antimicrobial activities [38]. Surface concentrations of AMPs up to 5.9 μg/cm^2 were achieved by conjugating the peptides to surface-immobilized primary amine functionalized polymer chains obtained by aqueous surface-initiated atom transfer radical polymerization of *N*,*N*-dimethylacrylamide and aminopropyl methacrylamide hydrochloride [38]. The efficacy of AMPs attached to catheter material surface using polymer brushes was verified *in vivo* by using a catheter-associated urinary tract infection mouse model [39]. By adding arginine–glycine–aspartate peptides to promote host-tissue cell adhesion to AMPs anchored through the block copolymer Pluronic F-127, two effects were achieved, namely thwarting bacteria from approaching and attaching to the surface and, simultaneously, enhancing tissue integration [40].

A completely different approach is given by immobilizing bacteriophages on surfaces [41]. Bacteriophages are viruses that infect bacteria and are highly efficient and relatively cost-effective. Bacteriophages are host specific, but they can have a broad host range, infecting several strains or species of bacteria, both Gram-positive and Gram-negative. They proved to be efficient in preventing bacterial contamination and recently they became accepted for food treatment to counter food contamination during storage. In addition, the fact that the EU is contributing €3.8 million to the Phagoburn study shows that it is willing to consider the approach [42]. Attachment of bacteriophages to a surface can be achieved through physisorption, electrostatic attachment, and covalent bonding [43]. Sample surfaces, which exhibited antimicrobial activities with immobilized phages, included gold, glass, cellulose membrane, and hydrogels [44–47]. Phages are specifically sensitive to moisture and can be deactivated when dried. However, reactivation upon wetting is feasible, and addition of polysaccharides improves their stability [43].

Two other groups of naturally occurring antimicrobials are claimed as alternatives to antibiotics: bacterial cell wall hydrolases (BCWHs) and antimicrobial peptides [48]. Antimicrobial peptides have a broad-spectrum against bacteria and fungus, low level of induced resistance, but may cause toxicity at high doses in order to be efficient and are more costly to produce. BCWHs have limitations towards Gram-negative bacteria, due to the presence of the outer membrane, and some important Gram-positive pathogens such as *S. aureus* are already resistant to lysozymes.

Biocide-releasing surfaces

Biocide-releasing surfaces may have some conceptual disadvantages since they are toxic by design in terms of releasing biocidal substances. In addition they will gradually become inactive and they may induce the formation of resistance [31]. Catalytically active surfaces, such as photocatalytically active surfaces (e.g. TiO_2) which regenerate reactive oxygen species upon UV radiation, provide an alternative.

Another approach is triggered release depending on certain threshold concentrations of quorum-sensing molecules which are found in biofilms [49,50].

Surface coating with carbon nanotubes (CNTs), graphene or diamond-like carbons (DLCs) promised interesting antimicrobial activity, since these materials show relatively low cytotoxicity towards mammalian cells. Whether these materials are active on the surface or whether they achieve antimicrobial activity through releasing traces into the aqueous phase is not yet resolved, but their activity in microbial suspensions is clearly demonstrated, e.g. higher toxicity is found for surfactant-dispersed CNTs [51]. The most frequently proposed mechanisms of action fall under four categories: (i) oxidative stress induction, (ii) protein dysfunction, (iii) membrane damage, and (iv) transcriptional arrest [52]. Recently, it was also demonstrated that the mechanism of action depends on the concentration of the bacteriocide – in this case graphene oxide (GO): low GO concentrations cut membranes of the micro-organisms *S. aureus* and *E. coli* whereas high concentrations induce the formation of GO aggregates shielding their edges. When cluster size increases, bacterial deactivation through wrapping is observed [53].

Graphene-based materials differ in their morphology (mono and multilayers) as well as in their surface chemistry (graphene, GO, reduced graphene oxide (rGO)). Lateral size for instance is important to enhance bacterial adhesion whereas the sharp edges may act as nanoknifes. GOs can enhance the antimicrobial activity through oxidative stress with or without the production of reactive oxygen species [54]. When comparing the antibacterial activity of graphite, graphite oxide, GO, and rGO towards *E. coli* under similar conditions, GO showed the highest antibacterial activity, followed by rGO, graphite, and graphite oxide [51]. Synergistic effects are reported for graphene-based silver nanocomposites and composites with other antibacterial nanoparticles, as well as with polymeric or enzymatic bactericides [55].

Carbon nanotubes have also been widely studied as antimicrobial material since they can be easily embedded into polymers. Again, a variety of morphologies has been studied such as single wall or multi-wall, but it seems that GO-based materials show higher antimicrobial activity [56]. Synergistic effects were obtained by making composites of CNTs and

chitosan within a hydrogel, or by decorating CNTs with poly(amidoamine)dendrimer-immobilized CDs and Ag_2S quantum dots which enhanced the antimicrobial activity in solution [57,58]. CNTs can also be used to prepare antimicrobial coatings either by electrodeposition of a polyvinyl-*N*-carbazole–CNT film or by preparing spin-coated films [59]. In the same work, the antimicrobial activity of dispersed CNTs was studied and it was found that such antimicrobial activity depended on the degree of dispersions. Antimicrobial activity of CNTs depends also on the length of CNTs, as was shown for poly(lactic-co-glycolic acid)-embedded CNTs, where the shorter ones were more active [60].

Diamond-like carbons represent a further morphology of carbon materials. In contrast to graphite, graphene and CNTs, tetrahedrally structured amorphous carbons with C–C sp^3 bonding are dominating with a significant amount of C–H bonds. They can be prepared by chemical vapour deposition, e.g. on stainless steel surfaces, and they can be doped with known antimicrobial metals such as copper, silver, or platinum [61]. When comparing the antimicrobial activity of pure DLCs and germanium-doped DLCs, significant reduction in *Pseudomonas aeruginosa* biofilm formation was observed whereas these surface films showed no effect against Gram-positive *S. aureus* biofilms [61].

Carbon quantum dots (CDs) are a relatively new class of carbon materials which can be used for bacterial identification due to their tunable photoluminescence properties. CDs exhibit low toxicity and appreciable biocompatibility [62]. When decorating the surface of CDs with QACs or Ag NPs, it was possible to selectively attach C-dots to Gram-positive bacteria and to induce antimicrobial activity through the membrane-disrupting mechanism [62,63].

Photocatalytic oxidation is a possible alternative strategy for antimicrobial coatings in the hospital environment [64]. Due to the self-regenerating biocidal effect of the catalytically released reactive oxygen species, such surfaces remain active throughout their lifetime. Many of the reported surfaces contain the photocatalyst TiO_2, which generates highly active OH-radicals in the presence of water, oxygen, and UV-A light. These highly reactive OH-radicals are able to destroy bacteria [65]. Current research is focusing on shifting the photocatalytic activity of such coatings towards the visible light range, e.g. by adding silver nanoparticles which can act through their surface plasmon resonance effects, or molybdenum [66,67]. When incorporating a combination of photosensitive dyes such as Crystal Violet with the inherently antimicrobial ZnO nanoparticles into polymer surfaces, synergistic photocatalytic antimicrobial activity was reported. The polymers exhibited significant bacterial kills using typical white light sources of hospital environments within 1 h against Gram-positive bacteria and within 6 h against Gram-negative bacteria [68]. By combining a dye with Ag nanoparticles, bactericidal activity of the Ag nanoparticles could be enhanced under white light illumination. It is believed that the enhancement effect is due to an increase in bactericidal activity through the triplet state of the dye by biomolecular reaction rather than by enhancement of the concentration of reactive oxygen species [69].

Surfaces decorated with metal oxide Lewis acids such as MoO_3 or WO_3 have also shown a broad-band antimicrobial activity [70]. Their mechanism of action is based on the in-situ generation of H_3O^+ ions through the reaction with moisture from the air [71,72]. The resulting acidified surfaces have a pH of 4.5–5.5 and the H_3O^+ ions are able to diffuse through the cell membranes where they can distort the pH-equilibrium and transport systems of the cell [72,73].

Reduced toxicity and prolonged durability of the antimicrobial effect may also be achieved by the triggered release of biocidal molecules. Recent strategies are based on quorum sensing: quorum-sensing molecules (e.g. homoserine lactones for Gram-negative bacteria) enable bacteria to detect the presence of other bacteria and to communicate with them [74]. The concentration of quorum-sensing molecules increases with bacterial multiplication and at certain threshold concentrations the expression of many genes is affected, such as genes encoding for adhesion or lipases, which are particularly abundant at sites of infection [75,76]. By coupling the antibiotic ciprofloxacin through a lipase-sensitive homoserine group on to the surface of a PEG model compound, a self-regulating system was obtained [75]. Alternatively, anti-quorum sensing enzymes could prevent bacteria from forming biofilms by suppressing the quorum-sensing molecule concentration below the threshold value [74].

Many plant extracts are well known for their antimicrobial properties and much research is devoted to their application to protect food from pathogens [77]. However, limited research has been done on investigating their efficacy on surfaces of healthcare units or on medical devices, such as tympanostomy tubes [78]. It has been shown that a tea-tree oil coating may induce zones of inhibition against MRSA after a two-day incubation [79].

Impact of topography on surface effectiveness

It is generally acknowledged that defects or design features on any inert surface can retain soil and/or micro-organisms, and therefore affect cleanability, disinfection, and hygienic status of the surface. Implications in the clinical environment in terms of cross-infection control, the choice of surface material to be used, and the cleaning and sanitization protocols are significant.

However, the assumption 'the rougher the surface, the worse the hygienic status' is somewhat simplistic, although many publications make this type of claim. Cells are easily removed from 'smooth' surfaces, but they may be retained within features approximating in size to that of the cells. In larger features, the cells may again be relatively easily removed. Typically, surface topography is measured by the R_a-value, defined as 'the average departure of the surface profile from a center line'. Other parameters are also used, but the R_a-value is the most popular in the microbiology literature. An R_a-value of 0.8 μm is often deemed indicative of a hygienic surface. In profilometry, a trace is taken of the surface, typically perpendicular to the lay of the surface features, using a probe. However, the probe will itself vary in size, depending on the method used to assess topography, from solid stylus, through laser scanning to the nanoscale tip of the atomic force microscope (which generates R_a-values in nanometers). The resolution of these different probes will affect the result obtained, although, using standardized surfaces of different degrees of roughness, the ranking (if not the absolute measurement) would likely be the same irrespective of the method used. In addition, the profilometer might reveal a two- or three-dimensional impression of the surface. If two-dimensional, then the overall picture

of the surface is not revealed: a feature identified along a linear trace might indicate a scratch or a pit — the type as well as degree of roughness may be important in terms of microbial retention. Three-dimensional images are more valuable, for example revealing very different topographies for surfaces with comparable R_a-values.

Since the R_a-value is a statistical measure, then the actual feature size, or the variation in feature size across a surface, is not revealed. This may be important if features of the dimension of microbial cells are present within larger features, with macro-, micro- and nano-features each potentially having different effects on the retention of cells on the surface. Indeed, the previously mentioned 'lotus effect' reveals that a hierarchical micro/nanostructure can significantly reduce retention, enabling cells to 'roll off' the surface. The fabrication of surfaces with well-defined nano-topographies provides a new avenue for the design of anti-adhesive/easily cleanable (and therefore hygienic) surfaces — depending on the intended environment of use [80,81].

The environment in which surfaces are placed will also affect their hygienic status. At a flowing solid–liquid interface, cells will move across the surface, and may be retained in features where they may replicate and form biofilms with accompanying 'streamers' which may detach and contaminate downstream [82]. This is a particular issue with joints in pipework. However, on open surfaces, at a solid–air interface, the cells tend to be deposited on the surface through contact with vectors such as food, fingers, equipment, or splashing [83]. In this case, replication is less likely, since water availability is low, and the survival of cells on these surfaces is key to maintaining hygienic status [84]. Antimicrobial surfaces, and/or surfaces which are hard or difficult to abrade, coupled with effective cleaning regimes, are strategies employed to counter this phenomenon. The continued cleaning/soiling cycle can itself affect the surface, causing abrasions that result in increased soiling and require increasing force in cleaning — which in turn may increase abrasion. The nature of the surface itself can affect how it wears: steel and other metals tend to scratch; glass and ceramics tend to fracture; softer materials such as plastics will abrade more easily.

Antimicrobial surfaces that actively leach out active agents might prove more effective if the surface area is increased through abrasion, but the presence of retained organic material (blood, food, sputum) in addition to micro-organisms might impede the antimicrobial effect and protect the microbial cells. It might be argued that the increase in surface area presented by surfaces with increased roughness is the driver for the increased retention — but this has not been convincingly proven. The features themselves, in terms of shape, profile, and size clearly provide an increased area of contact for cells, enhancing their ability to remain on surfaces [85]. All of these issues should be considered when developing novel and effective antimicrobial surfaces, focusing on minimizing wear to maintain cleanliness and cleanability.

At the cellular level, several studies focus on the retention of cells on surfaces. The typical experiment involves the incubation of surface with cell suspension for a specified time-period (such as 1 h), rinsing and removal prior to staining retained cells, and quantifying the amount of retention (cell numbers per unit area, or area of microscopic field covered by cells). However, one might debate which is more desirable: high numbers of retained cells which are easily removed, in comparison to low numbers of retained cells which resist detachment. Here, issues of cell survival and inactivation are also important.

The atomic force microscope is one means of assessing the strength of attachment of cells on a surface. The probe scans repeatedly across the surface, moving vertically in response to surface features. This movement is captured and imaged using lasers. By increasing the force of the scan, less strongly attached cells are removed. Thus the strength of attachment as well as the amount of retention can be assessed [85]. This work has revealed that the size of cells and their relationship with the feature size affect strength of attachment: as might be expected, comparable feature size and cell size is the least desirable combination, enabling maximum contact area between cell and surface. In addition, cell shape will also affect this interaction, with rod-shaped cells having a larger area of contact available for interaction with the cell surface in comparison to cocci. Investigation of the strength of attachment of cells on linear features where the force is applied either across or along the feature has revealed different results: demonstrating easier removal along well-defined features on titanium-coated stainless steel, but easier removal by applying force across features on softer polymeric surfaces [86,87]. As noted above, this work has led to the fabrication of surfaces with designed topographies that are targeted at inhibiting attachment of particular cells, where surface features smaller than cells might reduce their ability to strongly attach to the surface, and therefore improve cleanability (Figure 2). The robustness of these surfaces is essential to ensuring a long-lasting effect, and the potentially interfering effect of organic material must also be considered.

When considering open surfaces that are usually present at a solid–air interface, which is the main focus of this paper, biofilms are of less concern. In the clinical/medical environment, high-touch surfaces (worktops, walls, door-handles, telephones, patient surrounds) are the prime focus for antimicrobial treatments and/or effective cleaning. Solid–liquid interfaces, where biofilms could form, would likely be encountered around taps, showers or drains. The topography of the surface underlying the biofilm does not necessarily influence the quantity of the biofilm itself — again depending on the scale of feature size — but after cleaning, the substratum will retain cells in features which can regrow and reduce the time taken for the biofilm to develop once more.

Importance of safe-by-design in antimicrobial coatings

Application of the SbD approach for AMCs that are based on chemicals and nanomaterials is more challenging compared to

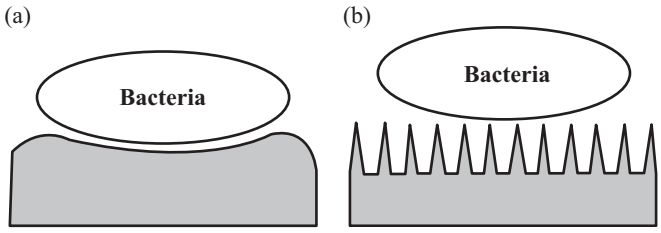

Figure 2. (a) Micron-sized features may favour bacterial adhesion, whereas (b) nano-sized features may create difficult topographic conditions for attachment.

those based on other types of materials, since usually there are neither standard test protocols nor enough data on the toxicity, absorption, metabolism, excretion, accumulation and biopersistence of these antimicrobial agents (especially of the nanomaterials), nor on their penetration into biological barriers both at an environmental level and mammalian cell level. EU programmes such as NanoFase, SafeNano, ProSafe, NaNoReg, and Euro-NanoTox aim to allow the early assessment of the toxicity and fate of nanomaterials with a strategy to establish and implement standardized toxicological measurement, establish international standards and to provide centralized nanotoxicology information (Figure 3).

There are four main challenges in designing safe AMCs: (i) the toxicity of the materials used; (ii) the potential impact of the antimicrobial agent on the development of antimicrobial resistance; (iii) the durability of the antimicrobial activity in the long term (the long-term stability); (iv) the lack of standardized methods for testing the performance of the AMCs under representative environmental conditions, namely their antimicrobial efficacy and long-term stability.

Silver is the dominant type of antimicrobial agent used in AMCs, followed by zinc oxide, zirconium, zinc omadine, titanium dioxide, quaternary ammonia compounds. The current AMC market mainly relies on the antimicrobial metal nanoparticles. Therefore, when talking about the toxicity of AMCs, metallic nanoparticles — mainly nano-silver — are the main subject. The environmental persistence and toxicity of biocides and antimicrobial nanomaterials represent a potential health and environmental issue. This may create a dilemma in the attempt to control HCAI through antimicrobial strategies. Because these antimicrobial strategies are employed to kill/control bacteria while they pose the risk of spreading, assessment is required for less known, less traceable and controllable nanoparticles with hardly understood mechanisms of action, toxicity and fate in the environment as well as within the body. One of the goals of the AMiCI group is to join forces to tackle such issues.

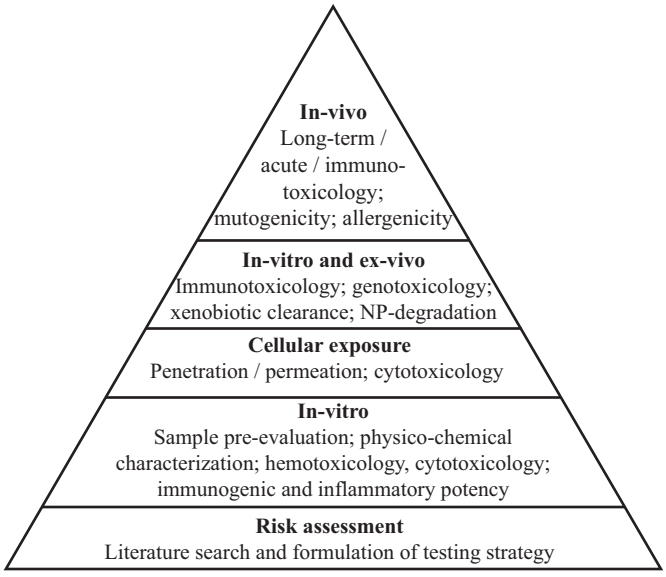

Figure 3. Multi-level Euro-NanoTox strategy. NP, nanoparticle. Adapted from Falk [12].

Mankind discovered the existence of one-celled microscopic creatures in the 1670s following the studies of Leeuwenhoek. Then it took almost 260 years to discover the first antibiotic for the control of bacteria, but much longer to develop techniques for the detection, monitoring and identification of bacteria in various environments. We are now equipped with advanced technology tools that enable us to detect and identify bacteria in minutes or even in seconds with 'standardized methods', as well as with many antibacterial agents effective in killing and controlling bacteria. However, it is hard to say that the human race won the fight against bacteria, as it is obvious that many of the agents discovered and used against bacteria resulted in the evolution of bacteria into much more resistant forms. Experts in this field and the Centers for Disease Control and Prevention define this problem as a 'nightmare' and an 'apocalyptic threat' for the human race. Although, shortly after his discovery of penicillin in 1928, Alexander Fleming warned about the development of antimicrobial resistance, it was not until the 2000s that outcomes of antibiotic resistance were clearly understood and large-scale official actions have been started. The difficulty in preventing the spread of antibiotic resistance stems from the widespread dissemination of antibiotics, and thus antibiotic resistance develops in a wide range of environments including hospitals, agriculture and food, community, soil, water resources and associated sites. Therefore, the development and implementation of SbD strategies is a key issue for the development of future generations of coatings for healthcare environments, such as nano-antimicrobial-based AMCs. An important requisite of AMCs is thus to minimize the risk of development of another 'apocalyptic threat' due to the spread of biocides and antimicrobial nanomaterials and the potential development of associated antimicrobial resistance and other toxicity issues while trying to control and prevent HCAI. Taking into account our current ability to detect, identify and monitor the nanoparticles and to extend our knowledge on the mechanisms of action, toxicity and fate of these nanoparticles in various environments, this might end up with a worst scenario.

An in-depth analysis of the toxicity and persistence of nano-antimicrobials in the environment is necessary to assess the safety of AMCs within the context of SbD. The rationale behind adding an antimicrobial agent to a specific surface should be balance between the potential impact of the antimicrobial agent on the emergence of resistant microbial strains and the impact of preventing the spread of the pathogenic microbial strains within the healthcare environment, and thus on the control of HCAI [88]. Several strategies have been employed to produce SbD reduced toxicity antimicrobial nanomaterials [89,90]. It is found that the concentration of nanoparticles to which the cells are exposed, the type of surface coating, the nature and extent of doping, and the aspect ratio of the particles make significant contributions to the cell toxicity of the nanoparticles [91]. Recent studies showed that Fe-doping is a possible safe design strategy for preventing ZnO toxicity in animals and the environment [92,93]. Moreover, polymer coating and modification with poly ethylene glycol (PEG) reduces the toxicity and cellular uptake of silver nanoparticles (Ag NPs) [94].

The time and cost constraints of the toxicity assessment methods represent another challenge for toxicity assessment of the nano-antimicrobials used in AMCs. Predictive nanotoxicology models appear as a feasible alternative for toxicity assessment [95].

The potential impact of AMCs on the development of antimicrobial resistance is a challenge that should be taken into serious consideration. Although the prevalence is still low, silver-resistant bacterial strains were found in hospital sewage systems [96]. Studies revealed the risk of both co- and cross-resistance to antibiotics and antimicrobials used in AMCs (biocides and metals) [97,98]. Therefore, these metal-based antimicrobials might contribute to the maintenance and spread of antibiotic resistance factors. Sütterlin *et al.* reported that silver resistance genes are widely represented in clinical isolates of the genera *Enterobacter* and *Klebsiella* in Swedish healthcare facilities [99]. Therefore, to avoid further selection and spread of silver-resistant bacteria with high potential for healthcare-associated infections, the use of silver-based products needs to be controlled and the silver resistance monitored.

Antimicrobial efficacy and long-term stability against the standard hygiene and sanitation protocols implemented in healthcare facilities are the main performance criteria for the AMCs aimed at reducing HCAI in healthcare facilities. Failure or weakness in the rigorous assessment of the coatings may result in unreliable performance. The lack of standard protocols for assessing the antimicrobial efficacy and long-term stability of the AMCs represents another challenge, which makes it impossible to establish a proper comparison between AMCs with different antimicrobial agents and processing technologies that are both in the market and in the R&D phase. In fact, issues with demonstration of functionality, efficacy, toxicity and potential risk of antimicrobial resistance development are one of the main market restraints for the commercialization of AMCs [10].

Conclusions

As mentioned above, an effective antimicrobial coating must achieve a multitude of characteristics: (i) be able to control the pathogenic population of a surface; (ii) be stable (mechanically, tribologically and chemically) in the wide range of hospital settings; (iii) minimize (eco)toxicological hazards and risks of antimicrobial resistance emergence; (iv) be affordable and easily implemented. Future technological developments should hence aim at tackling most, if not all, of these points. The ultimate goal of the antimicrobial coating, namely the prevention of thousands of deaths occurring as a direct consequence of HCAI in healthcare facilities, cannot be tackled by the coating alone. But a tremendous common effort involving coating technology providers, clinical and cleaning staff as well as the responsible handling of antibiotics — to name merely the clinical and agricultural sectors among many others — is required.

Regarding the ability to control the pathogenic population of a surface, very promising strategies have emerged. One of these is widely known as selective killing, or the ability of antimicrobial surfaces to target only those species that are deemed to cause a risk to patients or hospital staff. Strategies such as the use of quorum sensing at a threshold concentration to release an antimicrobial compound have recently appeared. Others, such as the modulation of the colonization consortia as a whole to inhibit the dominance of pathogens, in a strategy similar to the one used to control the human microbiome, should start appearing as microbial ecological concepts are better deciphered.

Depending on their intended use, antimicrobial surfaces will be challenged by a number of factors. For instance, door handles are in intermittent contact with hands, but nonetheless are not expected to be exposed to as much wear as bed linens, that should be washed on a daily basis. Studies on the weariness or robustness of the different materials under different conditions are available, but they require further methodological standardization to allow for a more meaningful interpretation of the results.

Biocidal chemicals used in antimicrobial coatings are inherently toxic; biocide-releasing surfaces are subjected to the Biocidal Products Regulation (BPR, Regulation (EU) 528/2012) and by the Regulation (EC) No. 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). This regulatory barrier makes it very expensive and time-consuming to bring antimicrobial coatings, of which the antimicrobial activity is related to biocidal release, to the market. On the other hand, anti-adhesive surfaces can be considered as SbD since there are no biocidal molecules involved which may either induce toxic effects or lead to the evolution of resistance. In this respect, the control of specific surface topography characteristics, in conjugation with chemically based strategies and specific cleaning protocols, could lead to a novel generation of improved SbD products.

Overall, the novel strategies that are continuously being developed in the area of nanosurfaces bring some hope to the field of antimicrobial control, while decreasing microbial resistance to antibiotics and associated infections in clinical settings. It is then crucial to provide suitable standardized assessment tests and a fast transition of these strategies from the lab bench to the market, by conjugating efforts between academia and industry.

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None declared.

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